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THE BACTERIOLOGY OF THE NORMAL CONJUNCTIVAL SAC, FROM A REPORT OF 200 CASES, AND ITS PRACTICAL BEARING ON THE UTILITY OF ANTISEPTICS IN OPHTHALMIC SURGERY.

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The bacterial condition of the normal conjunctival sac is a matter of general interest to ophthalmologists, not only from the merely scientific standpoint of ascertaining the variety and number of the contained bacteria, but also from its practical bearing on the methods of ophthalmic surgery.

If an apology is needed for this paper, it lies in the fact that the bacterial condition of the normal conjunctival sac has never been thoroughly worked out in this country, and, although a considerable number of investigations have been made in other countries, there has been so much diversity of opinion expressed, that a further contribution may help to a definite and satisfactory conclusion on this important subject.

I will, firstly, give a brief summary of the work of others before proceeding to the results of my own observations.

(1) There is a general consensus of opinion that the normal conjunctival sac contains bacteria in the majority of cases.

(2) Opinions differ as to whether the normal conjunctiva is ever sterile. Leber considered that it was frequently sterile.

Franke's[1] researches found the healthy sac sterile in 28 per cent. of cases.

More recently Morax has expressed his belief that the normal conjunctiva is never sterile, and in this he is supported by Randolph[1] in a very recent article upon the subject. The question largely turns upon the impossibility of testing the whole conjunctival surface, so that sterility in inoculated tubes cannot be held to afford complete proof of the sterility of the entire conjunctival sac.

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[1] Von Graefe's *Arch. of Ophth.* Bd. xxxix. Abth. iii.

(3) Most, if not all, of the later writers agree that the number of bacteria present in the healthy conjunctiva is small.

(4) As regards the pathogenic properties of the bacteria found, earlier observers agreed that the pyogenic cocci were frequently present. The latest researches go to shew, however, that the *staphylococcus epidermidis albus*, a comparatively inert form of *st. pyogenes albus*, very frequently replaces the latter microbe. Randolph[2] thinks that probably all bacteria found in the normal conjunctiva have but little, if any, pathogenic power.

PERSONAL OBSERVATIONS.

My own observations have extended over a series of 200 cases. They were undertaken at the Jenner Institute of Preventive Medicine, and I was much helped by the kind assistance and great experience of Dr. Allan Macfadyen, the Director of the Institute, and Dr. R. T. Hewlett, to both of whom I take this opportunity of tendering my most cordial thanks. I desire also to express my grateful thanks for the facilities offered me by the Institute.

The greatest care was taken to select cases for examination in which the conjunctiva was both subjectively and objectively normal, that is, smooth, of normal colour and consistence, with total absence of any smarting or pricking or morning gumming of the lids. Cases were further chosen with a view to difference in surroundings; thus, a certain proportion of cases were taken from people living in the country far away from any town, whilst others were taken from people living in London. As might be expected, there was a larger proportion of sterile tubes amongst the country than amongst the town cases.

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[1] RANDOLPH. *Arch. of Ophth.* Vol. xxvi. 3. 1897.

[2] *Ibid.*

The following method was employed to carry out the examination :—A platinum looped needle, after careful sterilisation in the flame, was rubbed well over the conjunctival surface of the lower lid and over the bulbar conjunctiva. The irritation caused by the presence of the wire soon induced a slight flow of tears, and thus a washing of the contents of the sac was made to pass over the needle. Care was taken to prevent any contamination of the wire from the edges of the lids or eyelashes, the experiment being repeated if such an accident occurred. A second inoculation was also made in cases where one was not deemed sufficient. As regards the use of the platinum wire loop as a means of testing the conjunctival surface, the chief advantage lay in its easy sterilisation. By forming a fair-sized loop, a large amount of the wire surface was exposed, and if many organisms were present it would be impossible for the wire to remain uninfected. The most perfect method would undoubtedly have been to employ a camel's hair pencil; but it would have presented considerable difficulties in the way of efficient sterilisation, and it was considered that the results obtained by the wire loop would give a sufficiently accurate estimate of the bacterial condition of the sac.

Further, the platinum loop has been the method usually adopted in other researches of this kind.

Loeffler's blood serum was the medium selected as the one most suitable for the primary inoculations. This medium also possessed a special advantage on account of the frequent appearance of the xerosis bacillus, an interesting fact to which I shall refer again.

The following results were obtained: — Two hundred primary inoculations were made on tubes of Loeffler's blood serum. One hundred and fifty-nine tubes presented growths after inoculation. Forty-one tubes, or 20·5 per cent., remained sterile after inoculation.

The most interesting growth from a bacteriological point of view was that of the so-called *xerosis bacillus*, which was found present in no less than 118, or 74·2 per cent., out of the 159 of the fertile tubes. In ninety cases, or 56·6 per cent. of the fertile tubes, this bacillus was found alone in pure culture, whilst in the remaining twenty-eight cases it was found mixed with other organisms. In each of these twenty-eight cases the xerosis bacillus was the predominating growth, and, with very few exceptions, the other micro-organisms were only present as one or two scattered colonies.

The following pyogenic cocci were isolated:—

The *st. pyogenes albus* was found in six cases, or in 3·7 per cent. of the fertile tubes, or 3 per cent. of the total 200 tubes. It was only found in pure culture twice. In the other four cases it occurred as one or two small isolated colonies in company with the xerosis bacillus.

The *st. pyogenes aureus* was found in one case. It occured in pure culture as a single small colony.

The *st. pyogenes citreus* was found in three cases, or 1·8 per cent. of the fertile tubes, or 1·5 per cent. of the 200 tubes. It was isolated in pure culture in one case, and then appeared as a fair sprinkling of small yellow colonies. In the remaining two cases it occurred as one or two colonies mixed with the xerosis bacillus.

The *st. cereus albus* appeared in four cases, or 2·5 per cent. of the fertile tubes, or 2 per cent. of the 200 tubes. In each of the four cases it grew as one or two small whitish colonies in company with the xerosis bacillus.

Fraenkel's pneumococcus was found in two cases. In both instances it appeared in pure culture.

Thus only sixteen tubes, or 8 per cent. of the 200 tubes, contained growths of the pyogenic cocci, and only in four tubes did they appear in pure culture. In every case, with the exception of one tube of st. pyogenes citreus, the number of colonies present were few and far between, being in some instances limited to a single colony. The pyogenic properties of the st. pyogenes citreus and the st. cereus albus, are comparatively slight, and, if we except these growths, we have only left seven tubes containing the st. pyogenes albus, and the st. pyogenes aureus, two of the most important varieties of the pyogenic bacteria.

In order to test the local virulence of the pyogenic organisms which were isolated, inoculations were made into the conjunctival sacs of guinea-pigs and rabbits, and in every case the result was negative and no reaction whatever occurred. The inoculations were made chiefly by injecting hypodermically into the conjunctival sac a powerful dose of the organism freshly grown in peptone broth. In a few cases another method was employed; viz., a platinum loop well smeared over with a 24-hours-old agar culture of the organism was well rubbed over the conjunctival surface. In one case of the st. pyogenes albus both methods were employed within four days of each other and fresh cultures were used in each case, but no reaction followed either inoculation. I regret to say that no inoculation was made with the st. pyogenes aureus, which was found in one case, but, having regard to the absence of results in experimenting with the other varieties of pyogenic cocci, I greatly doubt whether I should have met with any greater success with the aureus.

With reference to this point, it is interesting to note that Leber,[1] Weeks,[2] and others have inoculated the st. pyogenes aureus into healthy human conjunctival sacs without any result. No inoculations were made with the pneumococcus of Fraenkel. Both cases occurred in the same patient, and the growth died out in three days before I had an opportunity of testing its virulence.

So far as my investigations go, therefore, they point strongly to the fact that the pyogenic cocci only occur infrequently in the normal conjunctival sac, and that when found in this situation, they are either of a non-virulent type or, at any rate, speedily lose their pyogenic properties after their entry into the conjunctival sac.

The following variety of micro-organisms other than the pyogenic cocci and the above-mentioned xerosis bacillus were isolated:

The *st. epidermidis albus* was isolated in fourteen cases.

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[1] *Die Bedeutung der Bakt., &c.* Seventh Intern. Ophth. Cong., Heidelberg, 1888.

[2] *Arch. of Ophthal.*, vol. xvi, p. 376.

In ten cases it appeared in pure culture, whilst in four cases it occurred as a few small colonies mixed with the xerosis bacillus. From six of these cases fresh sub-cultures were made and inoculated into the conjunctival sacs of guinea-pigs after the methods above enumerated. In each case the result was negative. It is generally conceded that this coccus is a harmless, inert variety of the st. pyogenes albus, and, consequently I have not reckoned it with the list of the pyogenic cocci.

In twenty-one other cases various other non-pathogenic bacteria, resembling the ordinary saprophytes of air and water, were found. Eleven cases occurred in pure culture, whilst the remaining ten cases appeared as one or two colonies mixed with the xerosis bacillus. There were four or five varieties of these bacteria, and an inoculation of each variety was made into the conjunctival sac of a guinea-pig, without, however, causing any reaction.

Sarcina alba and sarcina flava were found in six cases. In three cases sarcina was the only growth isolated, and in the other three cases it occurred with the xerosis bacillus.

Two varieties of torula were found in one case as the only growth present.

In eight cases various non-pathogenic bacilli were encountered. These included two cases of a large sporing bacillus, four cases of a large bacillus liquefying blood serum, and two cases of bacilli belonging to the mesentericus group, viz., the bac. mesentericus ruber and fuscus. One case of the bacillus which liquefied blood serum was inoculated into the conjunctival sac of a guinea-pig, but without any result.

The chief features of this report which I wish to bring under notice are :—

(1) The frequency with which the so-called xerosis bacillus was found.

(2) The comparative infrequency of pyogenic micro-organisms and the non-virulent type presented.

(3) The considerable number of sterile tubes.

1st. *With regard to the xerosis bacillus.*

Although the bacillus has been found by Uhthoff as an occasional inhabitant of the normal conjunctival sac, yet other observers such as Franke and Eyre[1] have failed to isolate it. I believe that the frequency of its presence may have been overlooked because of the difficulty of growing the primary culture of the bacillus on any other medium than blood serum. For this medium the xerosis bacillus has the greatest partiality, and both primary and secondary cultures grow upon it with great freedom. Upon agar-agar it will seldom or never grow as the primary culture, and even secondary cultures form only a faint translucent film, whilst in gelatin the organism will never grow as a primary culture, and very rarely at all as a secondary culture.

The morphological resemblance of the xerosis bacillus to the Klebs-Loeffler bacillus makes it an easy growth to recognise, whilst the appearance of its colonies on blood-serum are quite characteristic. It is impossible in this paper to discuss the possible identity of the xerosis bacillus with the Klebs-Loeffler bacillus or the pseudo-diphtheritic bacillus, but the frequency with which I met this most interesting organism proves it to be the most common and universal resident of the normal conjunctival sac.

For a long time it has been gravely doubted whether this bacillus has any pathological connection with the disease known as xerosis. It is true that cases of this disease always show large numbers of this bacillus; but I have found many instances presenting just as free a primary growth of the bacillus in the healthy normal conjunctival sac as can be procured from cases of the disease itself. It is well known that the bacillus has no pathogenic properties in guinea-pigs and rabbits, and I have satisfied myself of the truth of this statement by inoculating fresh cultures into the conjunctival sacs of these animals.

The frequency with which this organism was encountered (118 cases, of 159 fertile tubes) proves conclusively that the bacillus can have nothing to do with xerosis. Little seems to be known of its presence in other parts of the body.

[1] *Journal of Path. and Bact.*, vol. iv., 1, 1896.

It has been found by Neisser in wounds and ulcers, but in no other situation has it been isolated in the numbers and frequency with which I have found it in the normal conjunctival sac. Its name, therefore, is most inappropriate, and I would suggest that some other more suitable term be found for it, such as the "*bacillus conjunctivæ communis.*"

Another point which the frequent appearance of this bacillus suggests is the possibility of mistaking it for the true Klebs-Loeffler bacillus in cases of membranous conjunctivitis when examination is being made for the latter. The morphological resemblance between the two when young and stained with methylene blue is very striking, and unless it is a skilled observer who undertakes the examination, or there is a good specimen of the true Klebs-Loeffler bacillus handy for the sake of comparison, the chances of error are very large, and, though I have no positive example, it seems possible that such an error is not infrequently made, and that cases of severe membranous conjunctivitis have been considered to be of diphtheritic origin, on account of the xerosis being mistaken for the Klebs-Loeffler bacillus. If the observer is not a practised person at the differential diagnosis, the only safe and certain method, and the quickest in the end, is to inoculate a recent pure culture of the suspected bacillus into some animal such as a guinea-pig. Death will result in a few hours if the case be one of true diphtheria.

Eyre, in his excellent monograph on the xerosis bacillus, states that the colonies of the primary culture do not appear visible for thirty-six to forty-eight hours after incubation. According to my experience this is somewhat too lengthy a period. In nine cases of pure xerosis the colonies were recognised and were very plainly visible twenty hours after the tube was placed in the incubator. In two other cases I noted the appearance of colonies twenty-two hours after incubation. Both were pure cultures of xerosis. In two or three of these eleven cases, in order to be quite certain of this point, specimens were stained, mounted, and recognised as undoubted xerosis under the microscope. On the other hand, I noted down in one case that no colonies were visible to the naked eye eighteen hours after incubation, and in a certain number of cases I was certainly unable to detect signs of growth twenty-two hours after incubation. All my cases, however, exhibited well-marked colonies thirty hours after incubation; so that I should say that the usual time that xerosis makes its first visible appearance as a primary culture lies between twenty to thirty hours. All secondary cultures appear somewhat earlier than this, and if blood-serum or blood-smeared agar be employed abundant growth will be always apparent in less than twenty-four hours.

As regards Neisser's[1] method of staining the Klebs-Loeffler bacillus, I have always found it answer in an equally admirable manner with the xerosis bacillus. It has been claimed for this stain that it will serve as a point of differential diagnosis between the xerosis and Klebs-Loeffler bacillus, in that the former does not react to the stain unless of twenty-four hours' growth or more; whereas the Klebs-Loeffler bacillus will exhibit the granules from the time of its earliest appearance. This has not been my experience, for I succeeded in obtaining very good granular staining by this method in one case of xerosis of twenty-two hours' growth. This was a primary culture, and the specimen was therefore secured at the very earliest appearance of the growth.

Lastly, Eyre tells us that the xerosis bacillus, when grown in nutrient broth, produces slight alkalinity of this medium, and that this may serve as a useful point in the differential diagnosis between the xerosis bacillus and the Klebs-Loeffler bacillus, which invariably produces an acid reaction. I have in several cases grown xerosis on litmus agar, but have never succeeded in obtaining the slightest alteration in colour, whilst the Klebs-Loeffler cultures always produce marked reddening of the litmus. This difference in the behaviour of xerosis and Klebs-Loeffler bacilli, when grown on litmus agar, will serve as a practical diagnostic test and be more easily recognised than their reactions when grown in broth.

2*nd and* 3*rd*.—In briefly discussing the second and third points, viz., *the non-virulence and comparative infrequency of pyogenic cocci and the large number of sterile tubes* (41 in 200), I shall endeavour at the same time to draw some definite conclusions with regard to the utility of antiseptics in ophthalmic surgery.

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[1] *Zeitschr. f. Hyg.*, xxiv. 1897.

It will be noted that the streptococcus, which is probably the most potent and dangerous of all the pyogenic cocci, was entirely absent. Nothing like it was isolated in any of the 200 cases. This experience endorses that of other observers, and I think there can be no doubt that this micro-organism is *never* found in the healthy conjunctival sac, and that thereby one considerable element of danger in dealing with wounds involving destruction of the conjunctival epithelium is eliminated.

Now, in trying to form an estimate of the danger of wounds of surface where pyogenic cocci may be found, there are a good many factors to bear in mind beyond the mere presence of such micro-organisms.

Pyogenic organisms are not capable of themselves of causing suppuration. There must be, in addition, certain favourable conditions, both as regards the tissue in which the microbes lie and as regards the bacteria themselves.

These conditions have been set forth, in a most explicit manner, by Mr. Watson Cheyne in his work on "Suppuration and Septic Diseases."

As regards the tissue, we have to consider conditions which may prejudice the general vitality of the patient or the local vitality of the part. Upon the former point I need say no more in this paper, but with reference to the latter it is important to note that this local vitality, or power of offering resistance to the growth of pyogenic organisms, varies greatly in different tissues of the body. Thus, the endothelial cells of the peritoneum exhibit this vitality in a marked degree, and present a strong contrast to the more feeble resistance offered by the connective tissue cells of synovial membrane.

I shall point out later that the conjunctival epithelium seems to possess a great local vitality, similar to that of the peritoneal epithelium.

As regards conditions affecting the bacteria themselves, we have to consider the important factors of dose and virulence. Mr. Watson Cheyne has pointed out, and shown by direct experiment, that the severity of the affection resulting from the introduction of pyogenic bacteria varies directly, within certain limits, with the amount of virus introduced. These certain limits are constituted by the susceptibility of man to the influence of the virus and by the general and local vitality of the tissues. As regards virulence, there can be no doubt that the virulence of pyogenic micro-organisms diminishes with their age; so that we have most to fear from organisms that have been recently introduced.

Let us now consider the condition of the conjunctival sac with regard to these three factors:—(1) The local vitality of the conjunctival membrane; (2) the dose; and (3) the virulence of the contained organisms.

(1) *The local vitality of the conjunctival sac.*—The conjunctival sac is constantly exposed to contamination from without by particles of dust and all varieties of extraneous material. It is also in constant danger of infection from the nose by means of the lacrymal passage. Pyogenic organisms which have thus obtained an entrance are carefully fostered in its recesses by its peculiar anatomical conformation. For these reasons the conjunctival sac has always, until recently, been regarded as a species of anatomical sink—a nidus for any passing microbe. The reverse is the actual case. Instead of teeming with all manner and conditions of micro-organisms, the conjunctival sac is singularly devoid, at any rate, of living bacteria. Forty-one of two hundred tubes were found absolutely sterile, and many more only presented a few discrete colonies. The conjunctival sac may possibly never be quite sterile, although, for my own part, I believe that this is frequently the case; but at least the sterility of these forty-one tubes is strong evidence that living micro-organisms were few and far between. Indeed, one of the most striking features of my own observations was the small number of colonies in the large majority of the tubes; the only micro-

organism that was often plentiful being the so-called non-pathogenic xerosis bacillus. It is to be further remarked that other observers, such as Leber, Fick, and others, have failed to obtain growths of any description in a certain number of inoculations from the healthy conjunctival sac. In the face of these facts one cannot but believe that the conjuctival epithelium exerts a remarkable and detrimental influence upon the life and growth of bacteria.

(2) and (3) *Dose and Virulence.* If we pass on to the consideration of dose and virulence we obtain still further evidence of the great local vitality of the conjunctival membrane. In spite of its constant exposure to infection, pyogenic organisms of any description, including the st. epidermidis albus, were only found in thirty tubes. In almost every case the number of colonies was very limited. It is possible, and I do not wish to dispute it, that pyogenic organisms would have been found in all the 200 cases could the bacterial examination have been made absolutely complete, but the fact that none were found in 170 cases, and, if we exclude the st. epidermidis albus, in 184 cases, points to the conclusion that, if present, they were very few in number. Consequently we may conclude that in the healthy conjunctival sac we have only to deal with a very small number of pyogenic bacteria. Further, the negative results of inoculation of fresh cultures of these bacteria into the conjunctival sacs of guinea-pigs and rabbits showed that these bacteria had, in a great measure at least, lost their virulence.

The sum of these facts seems in short to warrant the conclusion that the healthy conjunctival saç, probably chiefly by its epithelium, but also possibly in part by means of the lacrymal secretion, is remarkably resistant to the growth and life of pyogenic bacteria, and, as a further deduction, that wounds of the healthy conjunctiva are not nearly so liable to suppurative processes as similar wounds in many other parts of the body.

Finally, let us briefly consider the bearing of these results upon the question of antiseptics in ophthalmic surgery.

In the first place, I doubt if any other branch of surgery can show such brilliant results as regards the general absence of suppuration as ophthalmic surgery. Suppuration in an eye after a well-performed operation with a normal conjunctiva is a very rare occurrence. It is not indeed the frequency of suppuration, but the hopelessly disastrous results to an eye if suppuration occurs, that makes the question of its exclusion of paramount importance, and has raised the question of the utility of antiseptics to one of the highest moment. In many other branches of surgery moderate suppuration prejudices and delays healing, but may not ultimately affect the successful result. With the eye it is different, and the onset of suppuration is the most dreaded misfortune that can befall the ophthalmic surgeon.

Secondly, as a result of much discussion and numerous papers, it has been generally conceded that antiseptics of such strength as to act as thoroughly effective germicidal agents are too irritating and, by their action in lowering the local vitality of the part, productive of more harm than good. Thus it has come about that a large number of the most experienced ophthalmologists have entirely discarded the use of such agents as mercury in their practice, preferring the risk, if any, that may be entailed by their avoidance to the prejudicial effects observed as their consequence.

The abolition, therefore, of antiseptics in modern ophthalmic surgery has its *raison d'être*, not so much from a disbelief in the efficacy of antiseptic solutions nor in a disregard of the principles which promote their use, but in a justifiable dread that any advantages they may possess are counterbalanced by still greater disadvantages.

The bacterial condition of the healthy conjunctival sac, as I have already detailed it, gives us, I venture to think, a further and still more reasonable ground for the abolition of antiseptic solutions in the treatment of eye wounds. We have, in short, in the conjunctiva, a membrane that is working on the surgeon's behalf, that possesses inherent antiseptic qualities, and nothing but harm can result from the use of any chemical agent which depresses and lowers its vitality, and thereby renders it less resistant to the growth of pyogenic organisms. The very fact of the exemption from suppurative processes that eye surgery has always enjoyed is in itself a strong proof of the antiseptic nature of the conjunctiva. Without doubt, the number of cases of suppuration has been still further reduced with the recognition of the importance of asepsis, but before the days of asepsis eye surgery always enjoyed great immunity in this respect. This is the more remarkable when we consider that if an eye is bandaged after operation, as is commonly done, any bacteria that may happen to be in the conjunctival sac are brought into close and constant contact with the wound service, whilst there still remains the open channel of infection from the nasal fossa by means of the lacrymal passage.

Thus, I would base and urge the abolition of antiseptics in ophthalmic surgery on the ground that the strongest antiseptic is the healthy conjunctiva itself, and in order to eliminate suppuration as far as possible from the list of accidents that may occur after operations we have only two factors to consider: (1) Perfect asepsis on the part of the operator, his instruments, dressings, &c., and (2) the healthy condition of the conjunctival sac.

Enough I hope has been said to show that we may part with antiseptics in ophthalmic surgery, not with an uneasy feeling that we had better be without them on account of the local disturbance that they produce, but with the satisfactory conviction that we do not need them on account of the antiseptic qualities of the conjunctiva itself.

I should add that the substance of this paper was read at the Edinburgh Meeting of the British Medical Association in 1898.